

Amendments to the Claims

Claim 1 (Currently amended):      A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked by a 5' splice acceptor sequence and a 3' splice donor sequence, wherein the exon marker sequence in a 5' to 3' direction contains:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end of the marker exon; and

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA downstream of the 3' end of the marker exon;

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with one or more Type IIS restriction enzymes that recognize one Type IIS RER site located at the 5' end of the marker exon and one Type IIS RER site located at the 3' end of the marker exon so that the cDNA upstream of the 5' end of the marker exon and downstream of the 3' end of the marker exon is cleaved, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream and downstream cellular exon tags;

subjecting said cDNA fragment to a ~~DNA polymerase~~ Klenow enzyme and nucleotides to generate a blunt-ended fragment;

self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;

amplifying a region of the cDNA fragment containing the exon tags in opposing orientations thereby generating a linear DNA molecule containing the exon tags in opposing orientations flanked by sequences corresponding to the marker exon 5' and 3' ends; subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzyme, thereby generating a linear DNA fragment containing upstream and downstream exon tags fused in an inverted conformation; cloning the fragments comprising the tags in inverted conformation; obtaining the nucleotide sequence of the cloned tags; and comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 2 (Original): The method of claim 1 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 3 (Original): The method of claim 1, wherein the polynucleotide construct is contained within a vector.

Claim 4 (Original): The method of claim 3, wherein the vector is a viral vector.

Claim 5 (Original): The method of claim 4, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 6 (Original): The method of claim 5, wherein the viral vector is a retroviral vector.

Claim 7 (Original): The method of claim 1, wherein the marker exon marker sequence encodes a fluorescent protein.

Claim 8 (Original): The method of claim 7, wherein the fluorescent protein is green fluorescent protein.

Claim 9 (Previously presented): The method of claim 8, wherein the fluorescent protein is detected and measured by flow cytometry.

Claim 10 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked by a 5' splice acceptor sequence and 3' splice donor sequence, wherein the exon marker sequence comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the marker exon

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker exon;

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA using a primer of sequence complementary to the sequence of the marker exon;

extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide sequence using a single deoxynucleotide triphosphate and an enzyme terminal transferase;

synthesizing a second and complementary cDNA using a DNA polymerase and a primer complementary to the homopolymeric sequence;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes ~~enethe~~-Type IIS RER site located at the 5' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream cellular exon tags with a 5' single-stranded overhang;

ligating a linker to the cDNA fragment, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment;

amplifying the linker and cDNA fragment with primers complementary to the marker exon and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme;

cloning the fragments;

obtaining the nucleotide sequence of the cloned tags; and

comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 11 (Original): The method of claim 10 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claims 12-14 (Cancelled).

Claim 15 (Original): The method of claim 10, wherein the polynucleotide construct is contained within a vector.

Claims 16-17 (Cancelled).

Claim 18 (Original): The method of claim 15, wherein the vector is a viral vector.

Claim 19 (Original): The method of claim 18, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 20 (Original): The method of claim 19, wherein the viral vector is a retroviral vector.

Claim 21 (Original): The method of claim 10, wherein the marker exon sequence encodes a fluorescent protein.

Claim 22 (Original): The method of claim 21, wherein the fluorescent protein is a green fluorescent protein.

Claim 23 (Previously presented): The method of claim 22, wherein the fluorescent protein is detected and measured by flow cytometry.

Claim 24 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the polynucleotide sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked at its 5' end by a splice acceptor sequence and at its 3' end by a splice donor sequence, wherein the exon marker sequence comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end of the marker exon; and

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker exon[[,]] ;

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA;  
synthesizing a second complementary strand of cDNA with a DNA polymerase enzyme and a primer whose sequence corresponds to the sequence of the marker exon;  
subjecting the cDNA to a Type IIS restriction enzyme that recognizes ~~one~~the Type IIS RER site located at the 3' end of the marker exon and thereupon cleaves the cDNA downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon and portions of downstream cellular exon tags with a 5' single-stranded overhang;  
ligating a linker to the cDNA fragment comprising the marker exon fused to a downstream flanking cellular exon tag, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment;  
amplifying the cellular exon tag with primers complementary to the marker exon and to the ligated linker;  
subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme used;  
cloning the fragments;  
obtaining the nucleotide sequence of the cloned fragments; and  
comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 25 (Original): The method of claim 24 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claims 26-28 (Cancelled).

Claim 29 (Original): The method of claim 24, wherein the polynucleotide construct is contained within a vector.

Claim 30 (Original): The method of claim 29, wherein the vector is a viral vector.

Claim 31 (Original): The method of claim 30, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 32 (Original): The method of claim 31, wherein the viral vector is a retroviral vector.

Claim 33 (Original): The method of claim 24, wherein the exon marker sequence encodes a fluorescent protein.

Claim 34 (Original): The method of claim 33, wherein the fluorescent protein is green fluorescent protein.

Claim 35 (Previously presented): The method of claim 34, wherein the fluorescent protein is detected and measured by flow cytometry.

Claim 36 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:  
introducing into the cell a polynucleotide marker fragment, the expression of which is obtained only upon integration of the polynucleotide marker fragment into an actively transcribing genome region of the cell, wherein such polynucleotide comprises in a 5' to 3' direction:  
two restriction enzyme recognition (RER) sites located at the 5' end of the fragment,  
wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the fragment; and  
two restriction enzyme recognition (RER) sites located at the 3' end of the fragment,  
wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the fragment;  
isolating mRNA from said cell;

reverse transcribing isolated mRNA into double stranded cDNA;  
subjecting the cDNA to digestion with one or more Type IIS restriction enzymes that recognize one Type IIS RER site located at the 5' end of the marker and one Type IIS RER site located at the 3' end of the marker fragment and cleaving the cDNA upstream of the 5' end of the marker fragment and downstream of the 3' end of the marker fragment, thereby producing a cDNA fragment comprising the marker fragment and portions of upstream and downstream cellular nucleotide sequence tags;  
subjecting said cDNA fragment to a Klenow enzyme and nucleotides to generate a blunt-ended fragment;  
self-ligating the cDNA fragment into a circular molecule, thereby fusing the two RNA nucleotide sequence -tags in opposing orientations;  
amplifying a region of the cDNA fragment containing the tags in opposing orientations, thereby generating a linear DNA molecule containing two tags in opposing orientations flanked by sequences corresponding to the marker fragment 5' and 3' ends;  
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzyme, thereby generating a linear DNA fragment containing two upstream and downstream tags fused in an inverted conformation;  
cloning the fragments comprising the tags in inverted conformation;  
obtaining the nucleotide sequence of the cloned fragments; and  
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 37 (Original): The method of claim 36 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 38 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:  
introducing into a cell a polynucleotide marker fragment, the expression of which is obtained only upon integration of the polynucleotide marker fragment into an actively transcribing



genome region of the cell, wherein the polynucleotide marker fragment comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the fragment, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the fragment;

two restriction enzyme recognition (RER) sites located at the 3' end of the fragment, wherein the RER sites are different from each other and, wherein ~~at least one of~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the fragment;

isolating mRNA from said cell;

reverse transcribing isolated RNA into single stranded cDNA using a primer complementary to the sequence of the marker fragment;

extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide sequence using a single deoxynucleotide triphosphate and an enzyme terminal transferase;

synthesizing a second and complementary cDNA strand using a DNA polymerase and a primer complementary to the homopolymeric sequence;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes ~~one the~~ Type IIS RER site located at the 5' end of the marker fragment and thereupon cleaving the cDNA upstream of the 5' end of the marker fragment, thereby producing a cDNA fragment comprising the marker fragment and portions of upstream cellular tags with a 5' single-stranded overhang;

ligating a linker to the cDNA fragment, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment;

amplifying the ligation products with primers complementary to the marker fragment and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER site not previously recognized by the Type IIS restriction enzyme used;  
cloning the fragments;  
obtaining the nucleotide sequence of the cloned fragments; and  
comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 39 (Original): The method of claim 38 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 40 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:

introducing into the cell a polynucleotide marker fragment, the expression of which is obtained only upon integration of the polynucleotide marker fragment into an actively transcribing genome region of the cell, wherein the polynucleotide marker fragment comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker fragment, wherein the RER sites are different from each other and, wherein ~~at least one of these~~ the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the fragment;

two restriction enzyme recognition (RER) sites located at the 3' end of the marker fragment, wherein the RER sites are different from each other and, wherein ~~at least one of the~~ RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker fragment;

isolating mRNA from said cell;

reverse transcribing isolated RNA into single stranded cDNA;

synthesizing a second complementary strand of cDNA with a DNA polymerase and a primer whose sequence corresponds to the sequence of the marker fragment;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes ~~one~~the Type IIS RER site located at the 3' end of the marker fragment and cleaving the cDNA downstream of the 3' end of the marker fragment, such that a cDNA fragment is produced comprising the marker, and portions of downstream cellular tags with a 5' single-stranded overhang; ligating a linker to the cDNA fragment, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment; amplifying the ligation products with primers corresponding to the marker and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme used;

cloning the fragments;

obtaining the nucleotide sequence of the cloned fragments; and

comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 41 (Original): The method of claim 40 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 42 (Original): The method of claim 36, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 43 (Original): The method of claim 37, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 44 (Original): The method of claim 38, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 45 (Original): The method of claim 39, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 46 (Original): The method of claim 40, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 47 (Original): The method of claim 41, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claims 48-67 (Cancelled)

Claim 68 (Original): A polynucleotide construct comprising:  
a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and  
wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one of the 5' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and  
wherein the marker exon contains at least two RER sites at the 3' end of the marker exon, wherein at least one of the 3' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and  
wherein said restriction recognition sites are located close from the border of the marker exon such that after cutting flanking exons generate sequence tags of at least 8 nucleotides.

Claim 69 (Original): A vector comprising the polynucleotide construct of claim 68.

Claim 70 (Original): A polynucleotide construct comprising:  
a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and  
wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and

wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end the marker exon.

Claim 71 (Original): A vector comprising the polynucleotide construct of claim 70.

Claim 72 (Original): A polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and

wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 3' end of the marker exon, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and

wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end the marker exon.

Claim 73 (Original): A vector comprising the polynucleotide construct of claim 72.

Claims 74-75 (Cancelled).

Claim 76 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:

a splice acceptor sequence;

a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA fused upstream of the 5' end of a marker exon;

a restriction enzyme recognition site;

a marker exon;

a restriction enzyme recognition site;

a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving sequences located downstream of the 3' end of the marker exon; and

a splice donor sequence;

isolating ~~said~~ RNA from said cell;  
reverse transcribing the isolated mRNA into double stranded cDNA;  
subjecting the cDNA to digestion with Type IIS restriction enzymes that recognizes the Type IIS  
RER sites located at the 5' and 3' ends of the marker exon and cleaving the cDNA  
upstream of the 5' end of the marker exon and downstream of the 3' end of the marker  
exon, thereby producing a cDNA fragment comprising the marker exon, and portions of  
upstream and downstream cellular exon tags;  
subjecting said cDNA fragment to a Klenow enzyme and nucleotides to generate a blunt-ended  
fragment;  
self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;  
amplifying a region of the cDNA fragment containing the exon tags in opposing orientations  
thereby generating a linear DNA molecule containing the exon tags in opposing  
orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;  
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the  
RER sites not previously recognized by ~~the first~~ Type IIS restriction enzymes, thereby  
generating a linear DNA fragment containing upstream and downstream exon tags fused  
in an inverted conformation;  
cloning the fragments comprising the tags in inverted conformation;  
obtaining the nucleotide sequence of the cloned tags; and  
comparing the individual sequence tags or pairs of sequence tags to a sequence database such  
that the RNA transcript corresponding to the sequenced tags is identified.

Claim 77 (Currently amended): A method for elucidating a RNA transcription profile in a  
eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:

- a splice acceptor sequence;
- a restriction enzyme recognition site;
- a Type IIS restriction enzyme recognition site oriented so that it is capable of  
cleaving DNA fused upstream of the 5' end of a marker exon;
- a marker exon;

a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving sequences located downstream of the 3' end of the marker exon;  
a restriction enzyme recognition site; and  
a splice donor sequence;  
isolating said RNA from said cell;  
reverse transcribing the isolated mRNA into double stranded cDNA;  
subjecting the cDNA to digestion with Type IIS restriction enzymes that recognizes the Type IIS RER sites located at the 5' and 3' ends of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon and downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream and downstream cellular exon tags;  
subjecting said cDNA fragment to a Klenow enzyme and nucleotides to generate a blunt-ended fragment;  
self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;  
amplifying a region of the cDNA fragment containing the exon tags in opposing orientations thereby generating a linear DNA molecule containing the exon tags in opposing orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;  
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzymes, thereby generating a linear DNA fragment containing upstream and downstream exon tags fused in an inverted conformation;  
cloning the fragments comprising the tags in inverted conformation;  
obtaining the nucleotide sequence of the cloned tags; and  
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 78 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:  
a splice acceptor sequence;

a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA fused upstream of the 5' end of a marker exon;  
a restriction enzyme recognition site;  
a marker exon; and  
a polyadenylation sequence;

isolating said RNA from said cell;  
reverse transcribing the isolated mRNA into single stranded cDNA using a primer of sequence complementary to the sequence of the marker exon;  
extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide sequence using a single deoxynucleotide triphosphate and an enzyme terminal transferase;  
synthesizing a second and complementary cDNA using a DNA polymerase and a primer complementary to the homopolymeric sequence;  
subjecting the cDNA to digestion with at a Type IIS restriction enzyme that recognizes a Type IIS RER site located at the 5' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream cellular exon tags with a 5' single-stranded overhang;  
ligating a linker to the cDNA fragment, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment;  
amplifying the linker and cDNA fragment with primers complementary to the marker exon and to the ligated linker;  
subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme;  
cloning the fragments comprising the tags in inverted conformation;  
obtaining the nucleotide sequence of the cloned tags; and  
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.



Claim 79 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one the 5' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and wherein the marker exon contains at least two RER sites at the 3' end of the marker exon, wherein ~~at least one~~ of the 3' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and wherein said restriction recognition sites are located close from the border of the marker exon such that after cutting flanking exons generate sequence tags of ~~at least 8-20~~ nucleotides;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with Type IIS restriction enzyme that recognize one Type IIS

RER site located at the 5' end of the marker and one Type IIS RER site located at the 3' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon and downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream and downstream cellular exon tags;

subjecting said cDNA fragment to a Klenow enzyme and nucleotides to generate a blunt-ended fragment;

self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;

amplifying a region of the cDNA fragment containing the exon tags in opposing orientations

thereby generating a linear DNA molecule containing the exon tags in opposing orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;

subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzymes, thereby generating a linear DNA fragment containing upstream and downstream exon tags fused in an inverted conformation;  
cloning the fragments comprising the tags in inverted conformation;  
obtaining the nucleotide sequence of the cloned tags; and  
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 80 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 3' end of the marker exon, wherein ~~at least one~~ of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end the marker exon;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA;

synthesizing a second complementary strand of cDNA with a DNA polymerase enzyme and a primer whose sequence corresponds to the sequence of the marker exon;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one Type IIS RER site located at the 3' end of the marker exon and thereupon cleaves the cDNA downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon and portions of downstream cellular exon tags with a 5' single-stranded overhang;

ligating a linker to the cDNA fragment comprising the marker exon fused to a downstream flanking cellular exon tag, wherein said linker contains a 3' single-stranded overhang ligatable to said 5' single-stranded overhang of the exon tags of said cDNA fragment;

amplifying the cellular exon tag with primers complementary to the marker exon and to the  
ligated linker;  
subjecting the amplification products to one or more restriction enzymes that recognize the RER  
sites not previously recognized by the Type IIS restriction enzyme used;  
cloning the fragments;  
obtaining the nucleotide sequence of the cloned tags; and  
comparing the individual sequence tags to a sequence database such that the RNA transcript  
corresponding to the sequenced tags is identified.